Amendments to the Specification:

Please replace paragraph **[0006]** with the following amended paragraph(s):

NM 5/22/08

[0006] The keto group provides a unique chemical reactivity not present in the common twenty amino acids due to its ability to participate in addition reactions involving either the carbonyl group or the acidic Cα position. This group also provides an alternative to the natural amino acid cysteine for the selective modification of proteins with a large variety of chemical reagents. The reactive thiol group of cysteine has been extensively used to attach various biophysical probes to proteins. *See, e.g.,* Creighton, T. E. (1986) Methods Enzymol. 131:83-106; Altenbach, C., et al., (1990) Science 248:1088-92; Brinkley, M. (1992) Bioconjug. Chem. 3:2-13; Giuliano, K. A., et al., (1995) Annu. Rev. Biophys. Biomol. Struct. 24:405-34; Mannuzzu, L. M., et al., (1996) Science 271:213-6; Griffin, B. et al., (1998) Science 281:269-272; [[~~Llopis, J.,~~]] Wu et al., (2000) Methods Enzymol. 327:546-64; and, Gaietta, G., et al., (2002) Science 296:503-7. Unfortunately, the labeling of single cysteine residues is often complicated by the presence of more than one accessible cysteine residue in a protein, as well as exchange reactions of the resulting disulfide in the presence of free thiol. Therefore, the availability of a nonproteinogenic amino acid with orthogonal reactivity makes possible selective modification of protein in cases where a single cysteine cannot be selectively labeled, where two different labels are needed, and where a disulfide linkage may not be sufficiently stable. The carbonyl group reacts readily with hydrazides, hydroxylamines, and semicarbazides under mild conditions in aqueous solution, and forms hydrazone, oxime, and semicarbazone linkages, respectively, which are stable under physiological conditions. *See, e.g.,* Jencks, W. P. (1959) J. Am. Chem. Soc. 81, 475-481; Shao, J. & Tam, J. P. (1995) J. Am. Chem. Soc. 117:3893-3899.

Amendments to the Specification:

Please replace the paragraph [0091] with the following amended paragraph(s):

NM 5/22/08 **[0091]** Of particular interest in incorporating unnatural amino acids into proteins is to have the ability to incorporate a keto amino acid. The keto group provides a unique chemical reactivity not present in the common twenty amino acids due to its ability to participate in addition reactions involving either the carbonyl group or the acidic Cα position. The carbonyl group reacts readily with, e.g., hydrazides, hydroxylamines, semicarbazides, etc. under mild conditions in aqueous solution, and forms, e.g., hydrazone, oxime, and semicarbazone linkages, respectively, which are stable under physiological conditions. *See, e.g.,* Jencks, W. P. (1959) J. Am. Chem. Soc. 81, 475-481; Shao, J. & Tam, J. P. (1995) J. Am. Chem. Soc. 117:3893-3899. Through the keto amino acid, proteins can be selectively labeled with a wide variety of other hydrazide or hydroxylamine derivatives (including sugars, fluorescence labels, biotin derivatives, spin labels, metal chelators, crosslinking agents, polyethers, fatty acids, toxins, etc.). *See, e.g.,* the addition of saccharide derivatives through a keto amino acid, e.g., in U.S. patent number 6,927,042, ~~the application~~ entitled "Glycoprotein synthesis," ~~attorney docket number 54A-000610US~~ filed on October 15, 2003, which is incorporated by reference.

Please replace the paragraph [0098] with the following amended paragraph:

NM 5/22/08 **[0098]** Unnatural amino acid uptake by a cell is one issue that is typically considered when designing and selecting unnatural amino acids, e.g., for incorporation into a protein. For example, the high charge density of α-amino acids suggests that these compounds are unlikely to be cell permeable. Natural amino acids are taken up into the cell via a collection of protein-based transport systems often displaying varying degrees of amino acid specificity. A rapid screen can be done which assesses which unnatural amino acids, if any, are taken up by cells. *See, e.g.,* the toxicity assays in, e.g., the application entitled "Glycoprotein synthesis," U.S. patent number 6,927,042, ~~attorney docket number 54A-000610US~~ filed on October 15, 2003; and Liu, D.R. & Schultz, P.G. (1999) *Progress toward the evolution of an*

organism with an expanded genetic code. PNAS United States 96:4780-4785. Although uptake is easily analyzed with various assays, an alternative to designing unnatural amino acids that are amenable to cellular uptake pathways is to provide biosynthetic pathways to create amino acids *in vivo*.

Please replace the paragraph [0141] with the following amended paragraph:

NM 2/22/08 130

[0141] Proteins or polypeptides of interest with at least one keto amino acid are a feature of the invention. The invention also includes polypeptides or proteins with at least keto amino acid produced using the compositions and methods of the invention. One advantage of keto amino acids is that they can participate in a variety of chemical reactions. The carbonyl group reacts readily with, e.g., hydrazides, hydroxylamines, semicarbazides, and/or the like, under mild conditions in aqueous solution, and forms, e.g., hydrazone, oxime, and semicarbazone linkages, respectively, which are stable under physiological conditions. *See, e.g.,* Jencks, W. P. (1959), *supra*; Shao, J. & Tam, J. P. (1995), *supra.* Through the keto amino acid, proteins can be selectively modified or labeled with a wide variety of other hydrazide or hydroxylamine derivatives (including sugars, fluorescence labels, biotin derivatives, spin labels, metal chelators, crosslinking agents, polyethers, fatty acids, toxins, etc.), e.g., to produce probes of protein structure and function, to generate proteins with enhanced catalytic or therapeutic properties, or for the development of bioassays using either immobilized or soluble proteins. *See, e.g.,* the application entitled "Glycoprotein synthesis," U.S. patent number 6,927,042,~~attorney docket number 54A-000610US~~ filed on October 15, 2003. In certain embodiments of the invention, an excipient (e.g., a pharmaceutically acceptable excipient) can be present with the protein. Optionally, a protein of the invention will include a post-translational modification.

Please replace the paragraph [0184] with the following amended paragraph:

NM 2/22/08 170

[0184] *See also* corresponding application entitled "Glycoprotein synthesis" U.S. patent number 6,927,042~~attorney docket number 54A-000610US~~, filed October 15, 2003, which is incorporated herein by reference.

Amendments to the Specification:

Please replace the paragraph [0002], under the title STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT, with the following amended paragraph:

NM 5/22/08 **[0001]** The invention was made with United States Government support for a portion of the work described herein under Grant No. GM62159 from the National Institutes of Health, and support under grant DE-FG03-00ER45812 and grant DE-AC03-76SF00098 from the Department of Energy, and support under grant number N0001498D0402 from the Office of Naval Research. The United States Government has certain rights in the invention.